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CHEMICAL CHANGES IN WHEAT DURING GERMINATION

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(WITH PLATE XXVIII AND TWO FIGURES)

Introduction

The subject of germination has received much attention of late, and the literature is extensive. In general, however, investigations have been directed toward two phases of the subject: (1) the external factors necessary for and affecting germination, and (2) the chemical changes occurring within the various parts of the seed during the process. The earlier investigations of this second phase, to which fuller reference will be made later, dealt directly with the chemical changes occurring within endosperm or storage substances of the embryo as germination advanced; but as a rule each of these studies has been limited to the consideration of some one substance, and hardly any two investigations have dealt with the same kind of plant. More recent work bearing upon chemical phenomena has been directed toward the problem of delayed germination. The results as summarized by CROCKER (8) show that in most cases this delay is due either to conditions existing within the seed coat, or to a morphological or physiological immaturity within the embryo, leading in the latter case to phenomena of after-ripening. This investigation is an attempt to determine somewhat more comprehensively the principal chemical

changes occurring in a single kind of normally germinating seed, and thus to contribute to the facts of germination in general, and at the same time to provide a possible basis of comparison for some of the still unsolved problems of delayed germination and after-ripening.

Historical

DETMER (9) in 1880 presented a comprehensive summary of the work done up to that time by himself and others on the physiology of germination, and in the section dealing with the metabolism of storage substances he outlined the general facts in regard to the appearance, in various parts of the embryo, of starch, sugar, and nitrogenous compounds following the breaking down of reserve substances. BROWN and MORRIS (5), working on barley, found that the first visible change is the appearance of starch in the embryo, and localized the secretion of diastase in the epithelial cells of the scutellum. They also stated that the endosperm is a dead tissue incapable of self-depletion, although a few years later, after further work, BROWN and ESCOMBE (4) concluded that the aleurone layer is a living tissue by whose activity depletion of the endosperm might occur in the absence of the embryo. HANSTEEN (12) and PURIEWITSCH (17), however, maintained that the endosperm is capable of self-depletion provided the hydrolytic products are removed. The later work of Miss BRUSCHI (6) harmonizes these divergent views by showing that while self-digestion can occur in various kinds of grains, it does so to such different degrees that earlier investigators, working each upon a single form only, reached contradictory conclusions.

Of more direct bearing upon the subject is the work of LECLERC and BREAZEALE (14), in which by macrochemical analyses a quantitative study was made of the effects of different culture media upon the amounts of various organic and inorganic substances found in the several portions of the wheat seedling at different stages of germination. One of the latest contributions to the question of chemical changes during germination is that of Miss ECKERSON (11), who finds that in light-sensitive seeds active hydrolysis of hemicelluloses, fats, and proteins in the endosperm occurs in both light and darkness, but that in the light this process

begins toward the outside of the endosperm, the resulting substances diffusing out and away from the embryo, while in the dark it begins near the embryo, which can then make use of the hydrolytic products. The presence of iron in the seed coat, acting catalytically in the light, is undoubtedly a factor in the first case.

With the development of microchemical technique a method has become available by which qualitative determinations can be made for the presence or absence of many substances without an undue expenditure of time, and in this way, especially when checked with macrochemical analyses at crucial points, the time and place of chemical changes can be determined with greater accuracy than heretofore, as the following results show.

Method

The material used in this study was Marquis wheat, a hard spring wheat, procured from the Albert Dickinson Company of Chicago in the fall of 1917 and again in 1919. In most cases the ungerminated grains were soaked for two hours in distilled water in order to facilitate sectioning. No differences could be observed in the chemical condition of grains thus soaked as compared with unsoaked ones. For germinated material the grains were soaked for two hours and then placed in covered Petri dishes with moist filter paper on the bottom. These dishes were placed in a dark room kept at 16°–20° C., unless otherwise noted. When the period of germination covered several days the dishes were opened daily and the air renewed. Sections were cut freehand, and the microchemical tests employed were those recommended in the standard works by MOLISCH, TUNNMANN, and CHAMOT. A list of the tests used will be found at the end of this paper. Methods used in determinations other than microchemical ones are described in appropriate places. The germination period was regarded as seven days, in part because of the difficulty of growing seedlings longer under the given conditions, but mainly because by the end of that time the seedlings had so far developed that, had they been growing under field conditions, they would have been making their own food, without dependence upon the endosperm.

Microchemical study

UNGERMINATED GRAIN

COAT.—As the coat of the grain is easily permeable and in no way delays germination, no particular study was made of it. In general it consists of four layers: (1) the outer portion of the pericarp, consisting of one or more layers of cells whose walls contain some pectic substance, (2) the inner epidermis of the pericarp, in which lignification has occurred, (3) the testa, also lignified, and (4) the suberized remnant of the nucellar tissue. Glucose is present in the pericarp, undoubtedly a remnant of that originally present in the pericarp of the developing fruit (ECKERSON 10).

ENDOSPERM.—*Carbohydrates.*—Large amounts of starch are found in the endosperm, except in the aleurone layer, where it is entirely lacking. No reducing sugars are present, but a small amount of sucrose can be identified.

Fats.—Most of the cells of the endosperm contain very little fat, but it is abundant in the aleurone cells.

Proteins.—Proteins also are found in the starch-containing cells of the endosperm, and these are known to be almost entirely the storage proteins, glutenin and gliadin (OSBORNE 16). These cannot be distinguished from each other by microchemical methods. In the aleurone cells storage proteins are absent, but other protein material is present in considerable quantity.

Oxidizing enzymes.—No oxidase is found, but both peroxidase and catalase are present.

Minerals.—Little potassium is present, but considerable calcium and magnesium. Small amounts of phosphates were detected, while the aleurone layer has much iron. As noted under the embryo, no sulphur could be identified.

EMBRYO.—*Carbohydrates.*—Sucrose is the only storage carbohydrate found in the embryo of the Marquis wheat. In some forms, such as Emmer, starch is found in the scutellum.

Fats.—Fats are found in all parts of the embryo.

Protein.—The embryo gives a strong protein reaction, although probably no storage proteins are present. No amino acids can be detected by microchemical methods.

Oxidizing enzymes.—As in the endosperm, oxidase is lacking, but peroxidase and catalase are present.

Minerals.—Potassium and magnesium are present in considerable quantities. Calcium and phosphates could not definitely be identified, although undoubtedly present. Iron is found in abundance in the cells just under the epithelial layer. Sulphur could not be detected by any known microchemical methods.

Germinating grain

Carbohydrates.—No change is apparent in the contents of the grain (aside from the swelling due to absorption of water and softening of the tissues) until 10–12 hours after the material has been put into germinating dishes. At this time dextrin appears in the scutellum and coleorhiza, and starch in the root cap. At about the same time dextrin appears in the coleoptile and shortly afterward in the plumule. After 12 hours reducing sugar is found in the coleorhiza and appears also in the root, endosperm, coleoptile, plumule, and scutellum by the end of 24, 36, 48, and 96 hours respectively. After the appearance of the sugar in the coleorhiza and coleoptile, the amount of dextrin present decreases and the amount of sugar increases. In the root the amount of sugar increases up to the fourth day, after which the sugar content does not increase proportionately with the increase in the root tissue. At all times it is found most abundantly in the zone of the root hairs. In the endosperm reducing sugar is first found near the basal end of the embryo, but eventually is found throughout the whole tissue. All tests indicate that this reducing sugar is glucose. At the end of seven days starch is still present in the greatly disorganized endosperm, although practically all the grains still remaining show marked corrosion. A summary of this microchemical study will be found in table I.

The following quantitative study of the sugars in germinating wheat made by LeClerc and Breazeale (14) is of interest in checking up these microchemical findings. As the seedlings studied by these investigators were apparently grown in the light, photosynthesis may have influenced the result, although within the period of only seven days this would hardly be an appreciable

TABLE I

SUMMARY OF MICROCHEMICAL ANALYSES FOR DETERMINING PRESENCE OF STARCH AND REDUCING SUGARS IN GERMINATING WHEAT (16°-20°C.); ·+, PRESENT; ++, PRESENT IN ABUNDANCE.

HOURS IN GERMINATOR	LENGTH OF COLEOPTILE (MM.)	ENDOSPERM		SCUTELLUM		COLEORHIZA		ROOT CAP		ROOT TIP		ROOT		COLEOPTILE		PLUMULE	
		Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar	Starch	Reducing sugar
6.....	0*	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10.....	0	++	0	+†	0	+†	0	+	0	0	0	0	0	0	0	0	0
12.....	0	++	0	+	0	+†	0	+	0	0	0	0	0	+†	0	0	0
18.....	0	++	0	+	0	+†	+	+	0	0	0	0	0	+†	0	+†	0
24.....	0	++	+	+	0	+†	+	+	0	0	0	0	+	+†	0	+†	0
36.....	0	++	+	+	0	+†	+	+	0	0	0	0	+	+†	0	+†	0
48.....	2.7	++	+	+	0	+†	+	+	0	0	0	0	++	+†	+	+†	+
72.....	3.1	++	+	+	0	0	+	+	0	0	0	0	++	+†	++	0	+
96.....	6.6	++	+	+	+	0	+	+	0	0	0	0	++	+†	++	0	+
120.....	16.8	+	+	+	+	0	+	+	0	0	0	0	++	+†	++	0	+
144.....	32	+	+	+	+	0	+	+	0	0	0	0	++	+†	++	0	+
168.....	49	+	+	+	+	0	+	+	0	0	0	0	++	+†	++	0	+

* Plumule not well through coat until 36 hours.

† Dextrin.

factor. No statement is made in regard to the variety of wheat used. Table II is the summary of results, as given by LECLERC and BREAZEALE.

TABLE II

Part of plant and age	Reducing sugar as dextrose (mg.)	Hydrolyzable sugar as dextrose (mg.)
For 100 seeds		
Original seed	0	96
Seeds		
3 days	98.6	60
5 days	192.4	53
7 days	193.8	60
Axes		
3 days	148.0	50
5 days	253.7	42
7 days	267.9	46
Total plants		
3 days	246.6	110
5 days	446.1	95
7 days	461.7	106

Proteins.—Although both embryo and endosperm in the ungerminated grain give protein reaction, the storage proteins are known to exist only in the endosperm. During germination these are broken down, and at the end of seven days the nearly exhausted remnant of the endosperm gives only a very slight protein reaction. At this time, however, the aleurone layer is still intact, apparently unchanged.

No satisfactory microchemical tests are known for the derived proteins such as proteoses and peptones, so that no determination could be made for these substances. Some of the amino acids can be crystallized out of the tissue and the crystals identified by their chemical and optical properties. The first amino compound to be identified is asparagine, which was observed in the coleoptile on the fourth day and slightly later in the root. After the first appearance of asparagine in the coleoptile it accumulates there rapidly. A further discussion of the amino compounds in the seedling will be found in a later paragraph. At all times a marked protein reaction is obtainable in the stem and root tip, especially the latter (text fig. 1).

Oxidizing enzymes.—At no time was oxidase found in any part of the germinating seedling, but peroxidase and catalase were

present in all parts. In view of the increasing interest in the question of catalase activity, quantitative determinations were made on the seedlings on each successive day of the germination period. The method employed was that of APPLEMAN (1, 2) as used by JONES (13). In each instance three sets of 5 grains each were used. The air-dry weight of each set was determined; one set was then used for determining the final dry weight, and duplicate determinations were made on the other two, the results of which are given in table III. This shows that there is a marked and continuous increase in the catalytic activity of the seedlings during the first seven days of germination. Within the past few years investigations upon both plants and animals have shown a striking relation between the rate of catalytic activity and that of respiration. BURGE (7) showed a marked correspondence between the amount of catalase in different muscles of the body and the amount of work done by these muscles, and APPLEMAN (1, 2) has shown a direct increase in the catalase content in potatoes and corn with an increase in the respiration. RISCHAVI'S (18) standard work has given excellent data on the respiration of wheat as indicated by the release of CO_2 during a period of 21 days. Text fig. 2 shows the comparative curves of the rate of respiration as plotted from RISCHAVI'S figures for the first seven days, and that of catalytic activity during a similar period from the figures obtained as given in table III. As in the investigations cited, there is here a close parallel between respiratory activity and catalytic activity.

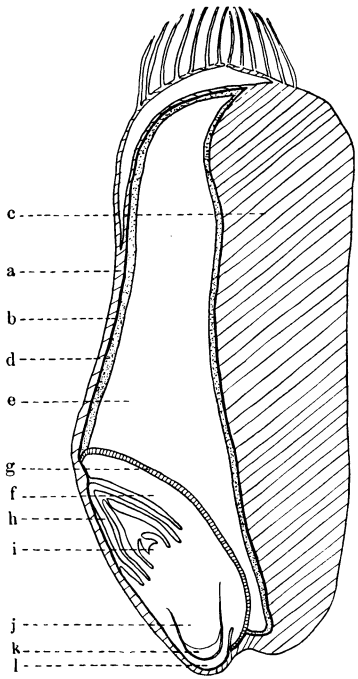

FIG. 1.—Longitudinal section of grain of wheat: *a*, pericarp and testa; *b*, suberized nucellar tissue; *c*, furrow in grain; *d*, aleurone layer; *e*, starchy endosperm; *f*, scutellum; *g*, epitheilal layer; *h*, coleoptile; *i*, plumule; *j*, hypocotyl; *k*, root cap; *l*, coleorhiza.

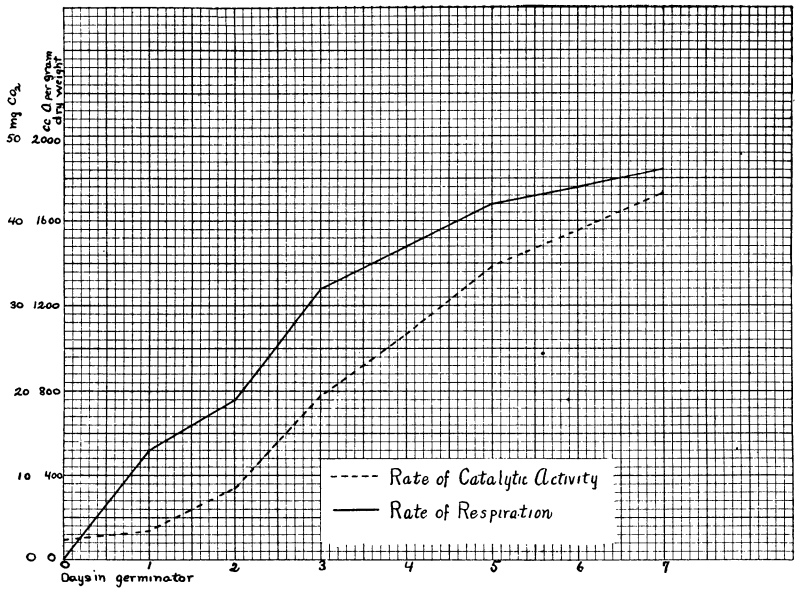

FIG. 2.—Relative rates of catalytic and respiratory activity (latter from RISCHAVI'S data).

TABLE III

CATALASE ACTIVITY OF GERMINATING WHEAT

Days in germinator	Original dry weight (gm.)	Final dry weight (gm.)	Oxygen (cc.) released in 10 min.	Oxygen (cc.) released per gm. of dry weight (calculated)	Average
0	0.146 0.1496	0.12848 0.1316	10 12	78 91	84.5
1	0.1406 0.1401	0.1251 0.12468	14.6 18.6	116 149	132.5
2	0.1482 0.1331	0.12967 0.11646	42.2 40	325 343	334
3	0.143 0.1446	0.1222 0.1236	93.4 93.6	764 781	772
4	0.1485 0.1438	0.1179 0.1141	122 127	1034 1113	1073
5	0.1286 0.120	0.10288 0.0768	126 119.6	1224 1570	1379
6*					
7	0.1582 0.134	0.11849 0.10036	198.4 178.6	1674 1779	1726.5

* Complete data lacking.

Increase in length of epithelial cells.—During germination there is a marked increase in length in the epithelial cells of the scutellum. In wheat this increase amounts at the end of seven days to 119 per cent in the distal cells, that is, those near the brush end of the grain; 161 per cent in the intermediate cells; and 165 per cent in the basal cells. The actual amount of increase in size is given in table IV.

TABLE IV

INCREASE IN LENGTH OF EPITHELIAL CELLS DURING GERMINATION

HOURS IN GERMINATOR	LENGTH OF EPITHELIAL CELLS IN MM.		
	Distal	Intermediate	Basal
0	0.0347	0.0346	0.0273
12	0.0388	0.0402	0.0305
18	0.0377	0.0377	0.0314
24	0.0407	0.0429	0.0340
48	0.0455	0.0568	0.0436
72	0.0573	0.0703	0.0518
96	0.0638	0.0821	0.0592
120	0.0629	0.0880	0.0562
144	0.0769	0.0899	0.0677
168	0.0762	0.0906	0.0725

From the physiological studies of BROWN and MORRIS (5) and such cytological work as that of TORREY (22), it was believed that these epithelial cells actually secreted the diastase used in the hydrolysis of the starchy endosperm, and that this increase in size accompanied an increasing secretion of diastase. Miss BRUSCHI (6), however, stated that while there is a marked increase in the size of the epithelial cells at this time, the hydrolysis of the starch is due, not to diastase secreted by them, but to that developed from a proenzyme existing within the amyliferous endosperm cells, and that it is the action of this enzyme which causes the self-depletion of the endosperm even in the absence of the scutellum, while the scutellum itself, when separated from the endosperm and grown under sterile conditions, produces no diastase.

Quantitative study of amino nitrogen content

The crystallization of amino acids from wheat by microchemical methods is difficult, probably because of the large amount of storage

protein present, and accordingly in order to obtain more exact knowledge on this point macrochemical analyses were made.

Miss ECKERSON (10) has shown that in ripening wheat asparagine, arginine, histidine, and leucine are present. As the formation of the proteins proceeds during desiccation, these amino acids disappear almost entirely, and only a trace of asparagine is left in the ripened grain. LEHMAN and OLTENWÄLDER (15) stated that unripened seeds frequently germinate more readily than wholly ripened ones because of the presence in the former of amino acids and active proteolytic enzymes, while in fully ripened seeds these enzymes which hydrolyze the storage proteins into more available forms are not always present in an active state. All investigations thus far indicate an increase in the amino acid content of seedlings during germination. Undoubtedly the most important work on this subject has been done by SCHULZE and his associates (19, 20) on the seedlings of *Lupinus luteus* and other leguminous plants, although in most cases on seedlings older than the wheat under consideration. In general he found that the first amino acids to appear are leucine, tyrosine, and the hexone bases, and concluded that the asparagine found somewhat later is a secondary product, formed from the mono-amino acids which serve as a storage substance to be used again in protein building. As the growth of the seedling advances, the asparagine content increases, while the amounts of the earlier formed acids decrease. The earlier theory of DETMER'S (9) that asparagine is a primary product of protein hydrolysis, and that its accumulation in seedlings grown in the dark is due to the absence of carbohydrates to unite with it to form new protein, seems improbable, as SCHULZE found almost as much in seedlings grown in the light as in the dark, and microchemical analysis clearly shows large quantities of sugar present in the coleoptile together with the asparagine in the seedlings over four days old.

The object of the analysis recorded in table V was not to isolate individual amino acids, but simply to determine the total amount of such substances. Determinations were made at three stages: (*A*) the ungerminated grain, (*B*) seedlings 3.5 days old, and (*C*) seedlings 6 days old. The temperature of the

dark room in which these seedlings were grown was 21°–23° C., which probably accounts for the greater length of coleoptile and root as compared with seedlings of the same age used in studying the carbohydrates. In sample *A* the air-dry wheat was finely ground in a food chopper, and in *B* and *C* the seedlings were cut up into small pieces with scissors immediately after removal from the germinating dishes. In all cases the material was preserved in 70 per cent alcohol. After extraction in hot alcohol

TABLE V

ANALYSIS OF MARQUIS WHEAT (1917) FOR AMINO NITROGEN CONTENT

Sample	Percentage	Percentage dry weight	Percentage soluble nitrogen
A (ungerminated)			
Moisture	10.78	.....	.....
Soluble nitrogen	.....	0.164	.....
Amino nitrogen	.....	0.0275	16.76
	Length of coleoptile 50 mm.		
B (germinated 3.5 days)			
Moisture	68.71	.....	.....
Soluble nitrogen	.....	0.837	.....
Amino nitrogen	.....	0.29	35.53
	Length of coleoptile 90 mm.		
C (germinated 6 days)			
Moisture	82.83	.....	.....
Soluble nitrogen	.....	1.66	.....
Amino nitrogen	.....	0.63	37.79

for six hours the material was ground in a mortar and a hot water extract made. As the presence of so much colloidal material (starch and protein) made it impossible to separate at all accurately the water from the solid matter, the entire mass was made up to 75 per cent alcohol, and all colloidal material precipitated by shaking with NaCl. The material was then filtered and the filtrate combined with the alcohol extracts and condensed in vacuo at 60°–70° C. to approximately 50 cc. This amount was then made up to volume (100 cc.) with distilled water. Determinations were made on this material for total nitrogen and

amino nitrogen. The former determinations were made by the BOCK and BENEDICT modification of the FOLIN-FARMER procedure (3), and this nitrogen is regarded as "soluble nitrogen" in table V. Determinations were made for amino nitrogen by the VAN SLYKE method. The results of these determinations are given in table V, from which it is seen that there is a considerable amount of amino nitrogen in the ungerminated grain, and that during germination this amount increases rapidly, while the increase in soluble nitrogen is less rapid. In comparison with these results, those obtained by THOMPSON (21) on the Alaska pea seedling are of interest. They are as follows:

PEAS	PERCENTAGE OF DRY MATERIAL	
	Total N	Amino N
Dry	....	0.088
3 days old	3.28	0.337
6 days old	3.48	0.747

It is also evident from the results given in table V that microchemical methods for identifying amino acids are not very satisfactory in the case of germinating wheat, probably for several reasons. The amino acids may be present in such small amounts that, although totaling an appreciable quantity, they cannot be detected individually; they may be those for which no satisfactory microchemical test has yet been found; or, as suggested earlier, other material present may prevent normal reactions from occurring. In the case of wheat such substances as storage proteins might easily interfere with the crystallization of the amino acids and so prevent their identification.

Summary

1. The principal storage carbohydrate of Marquis wheat is starch in the endosperm. A small amount of sucrose is also present in the endosperm and embryo.

2. The first noticeable chemical change during germination is the appearance in the scutellum and coleorhiza of dextrin, and in the root cap of starch. These substances appear simultaneously after about ten hours in the germinator (16°–20° C.). Later dextrin appears in the coleoptile and plumule.

3. Reducing sugar (probably all glucose) appears in the embryo after 18 hours in the germinator. It is first found in the coleorhiza, but soon afterwards appears in considerable quantity in all parts of the seedling, especially in the zone of root hairs and coleoptile.

4. During the germination period studied the increase in length of epithelial cells averaged 150 per cent.

5. Peroxidase and catalase are present in all parts of the grain both before and during germination. The amount of catalase present increases during the first seven days at a rate corresponding to the rate of increase in the respiratory activity.

6. During germination the protein content of the endosperm, except for that of the aleurone layer, decreases greatly.

7. Microchemical analyses show the presence of amino acids in the ungerminated grain and their increase in amount during germination. Microchemical analyses fail to indicate any amino nitrogen until the fourth day of germination. Asparagine is the only form that can then be so identified. This appears only in the root and coleoptile, accumulating in the latter in considerable quantity.

Microchemical tests

Pectic substances.—Ruthenium red, red color; methylene blue, violet color.

Lignin.—Phloroglucin and HCl, violet red color obtained without heating.

Suberin.—Insoluble in cold 50 per cent chromic acid.

Starch.—Iodine-potassium iodide, blue color.

Dextrin.—Amylo-dextrin, iodine-potassium iodide, red color; dextrin, precipitation of cuprous oxide upon long heating with Flückiger's reagent (see under fructose).

Fructose.—Flückiger's reaction; copper tartrate dissolved in 15–20 per cent NaOH, red precipitate obtained at once without heating.

Glucose.—Flückiger's reaction; red precipitate of cuprous oxide on heating 1–2 minutes; osazone crystals with phenyl-hydrazine reaction.

Sucrose.—First remove any fructose or glucose present as follows: apply Flückiger's reagent and, to remove any precipitate formed after heating for 2–3 minutes, wash with dilute tartaric acid solution, add warm concentrated magnesium chloride, and wash again in tartaric acid; then invert with invertase or dilute acid and test again with Flückiger's reagent.

Proteins (general).—Biuret reaction; xanthoproteic reaction.

Storage proteins.—Add $AlSO_4$ to form aluminium proteinate and then stain with logwood solution; not a specific reaction for individual proteins.

Amino acids and amides.—General test: Crystallize out in absolute alcohol; crystals of asparagine, glutamine, tyrosine, leucine, proline, and potassium nitrate may appear.

Specific tests: comparison with known crystal forms; observation with polarized light: asparagine, place sections in copper acetate, add absolute alcohol slowly and crystals of copper asparaginate appear; leucine, sublimation; arginine and histidine, picrolonic acid gives yellow crystalline precipitate.

Oxidizing enzymes.—Oxidase, blue color upon addition of guiaconic acid; peroxidase, blue color with guiaconic acid and H_2O_2; catalase, evolution of gas with addition of H_2O_2.

Minerals.—Calcium, with H_2SO_4 have calcium sulphate crystals; magnesium, formation of ammonium magnesium phosphate crystals; potassium, crystals of potassium-platinum-chloride upon addition of platinum chloride; iron, Berlin blue reaction (potassium ferrocyanide and HCl); phosphates, upon addition of magnesium mixture formation of ammonium magnesium phosphate crystals, upon addition of ammonium molybdate in nitric acid formation of ammonium phospho-molybdate crystals; sulphates, formation of benzidine sulphate crystals on addition of benzidine chloride.

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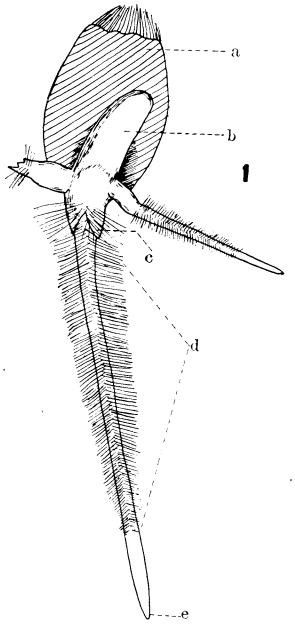

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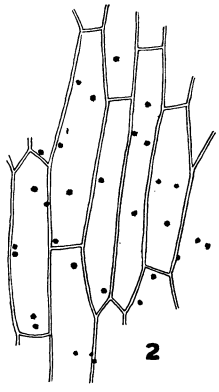

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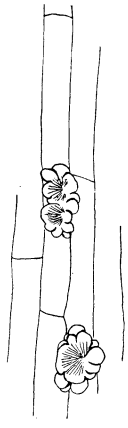

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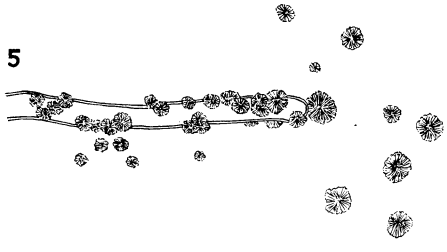

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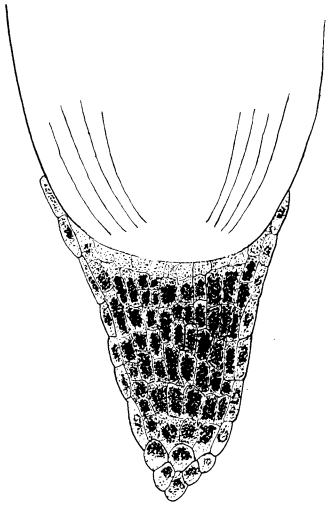

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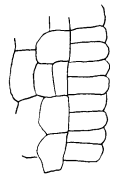

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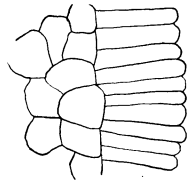

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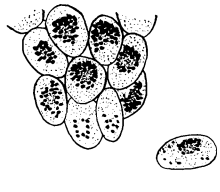

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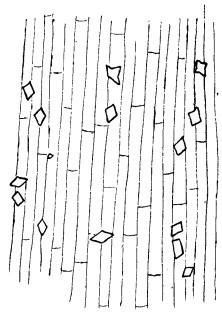

6

CHOATE on WHEAT

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EXPLANATION OF PLATE XXVIII

FIG. 1.—Wheat seedling, two days old: *a*, old kernel; *b*, coleoptile; *c*, coleorhiza; *d*, zone of root hairs; *e*, root cap; ×4.5.

FIG. 2.—Outer layer of pericarp showing cuprous oxide precipitated by Flückiger's reagent; ×150.

FIG. 3.—Root cap, showing starch stained with iodine (3 days old); ×112.

FIG. 4.—Detail of root cap; ×175.

FIG. 5.—Root hair showing crystals formed by osazone test; ×275.

FIG. 6.—Asparagine crystallized out in absolute alcohol in coleoptile (material 7 days old); ×45.

FIG. 7.—Copper asparaginate formed by addition of copper acetate and absolute alcohol in coleoptile (material 7 days old); ×150.

FIG. 8.—Epithelial cells of scutellum when placed in germinator; ×150.

FIG. 9.—Epithelial cells of scutellum after 7 days in germinator; ×150.